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Diagnostic value of the rectal ammonia tolerance test, fasting plasma ammonia and fasting plasma bile acids for canine portosystemic shunting

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ABSTRACT

Portosystemic shunting (PSS) often results in hyperammonaemia and, consequently, hepatic encephalopathy. This retrospective study evaluated the sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively) and other test performance metrics for the ammonia tolerance test (ATT), serum fasting bile acids (FBA), serum fasting ammonia concentration (FA), and combinations of these tests for their association with PSS in dogs. Medical records of 271 dogs suspect for PSS (symptomatic group) and 53 dogs returning for evaluation after surgical closure of a congenital PSS (CPSS post-surgical control group) were analysed.

In the symptomatic group, ATT at 40 min (T40), and the FBA had the highest sensitivity (100% and 98%, respectively) and NPV (100% and 96%, respectively) for PSS. The combination of increased FBA and FA had the highest specificity (97%), with a PPV of 97%, and a positive likelihood ratio of 29. In the CPSS post-surgical control group, the specificity and PPV of FA and the combination of increased FBA/FA were both 100%. In purebred populations, the NPV of all tests was 100%. Consequently, PSS would be ruled out in a symptomatic dog with normal FBA or ATT (T40) and would be highly probable when both FBA and FA are increased. Increased FA was conclusive for PSS in dogs evaluated for post-surgical closure of a CPSS. FBA was the most suitable test for screening purposes.

Introduction

Congenital portosystemic shunting (CPSS) or acquired PSS (aPSS), occurs when vascular anomalies that directly connect the portal venous system with the systemic circulation bypass the hepatic sinusoids (Van Den Ingh et al., 1995; Berent and Tobias, 2009). APSS develops secondary to portal hypertension as a compensatory mechanism to maintain normal portal pressure (Vitums, 1959); CPSS results from the persistence of embryonic portosystemic communications after birth and is classified as intrahepatic or extrahepatic (Van Den Ingh et al., 1995). Tobias and Rohrbach (2003) reported that the prevalence of CPSS was significantly higher in some dog breeds (0.1–3.2%) in comparison to the general population (0.05%).

Clinical signs of PSS are diverse and non-specific. Depression, ataxia, circling, convulsions and coma are associated with hepatic encephalopathy. Other clinical signs resulting from liver dysfunction, such as vomiting, diarrhoea, reduced appetite, polyuria/polydipsia (PU/PD), and lower urinary tract signs due to ammonia urate urolithiasis can also be observed.

Since the clinical signs associated with PSS are non-specific, sensitive and specific diagnostic parameters are required for further diagnostic workup. Serum fasting bile acids (FBA) and ammonia (FA) concentrations, and the ammonia tolerance test (ATT) are liver function tests that can be affected by abnormal portal flow to the liver (Meyer et al., 1978; Rothuizen and Van Den Ingh, 1982; Walker et al., 2001). Most studies that have assessed the diagnostic value of FBA and FA concentrations for the detection of PSS in dogs have only evaluated their sensitivity (Meyer et al., 1978; Center et al., 1985; Meyer, 1986; Johnson et al., 1987; Tisdall et al., 1994; d'Anjou et al., 2004). In those studies, the sensitivities of FBA and FA varied from 64 to 100% and from 81 to 100%, respectively. In two more recent studies (Gerritzen-Bruning et al., 2006; Ruland et al., 2010), the sensitivity and specificity of FBA and FA were calculated in large dog populations. In both studies the specificity of FBA was lower than FA. The diagnostic value of the ATT to detect PSS in dogs has been reported in a few studies, but none of these reported test sensitivity and specificity (Meyer et al., 1978; Rothuizen and Van Den Ingh, 1982; Meyer, 1986; Tisdall et al., 1994, 1995).

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The sensitivity and specificity of a test cannot be used to predict the probability of a disease in an individual animal. For this purpose, positive and negative predictive values (PPV and NPV, respectively) and positive and negative likelihood ratios (LR+ and LR–, respectively) can be useful when interpreting test results and deciding whether additional diagnostic tests are needed to confirm the diagnosis (Akobeng, 2007). Since disease prevalence influences PPV and NPV, the prevalence of a disease in the population of interest must always be taken in consideration when interpreting test results based on published test data.

The aim of this study was to evaluate the sensitivity, specificity, predictive values, likelihood ratios and accuracy of the ATT, FBA, FA concentrations and combinations of these tests for diagnosis and screening of canine PSS.

Materials and methods

Study design

Medical records were reviewed retrospectively for all dogs presented to the Department of Clinical Sciences of Companion Animals of the Utrecht University between January 2008 and January 2014. Inclusion criteria included an ATT and/or FBA and FA concentrations measured in the same blood sample; dogs were included only if PSS could be confirmed or excluded. Dogs were excluded from the study if they were not fasted at the time of specimen collection, or if the diagnosis of PSS was not confirmed/excluded.

Two dog populations were studied. The first comprised dogs with clinical signs suggestive of PSS presented for referral consultations (symptomatic group). The second population consisted of dogs that were presented for re-evaluation after surgical closure or attenuation of a CPSS (CPSS post-operative control group). Each population was divided into two groups: group 1 (PSS+) consisted of dogs in which PSS was confirmed. Group 2 (PSS–) consisted of dogs where PSS was absent. In the CPSS post-operative control group, dogs were evaluated 1 month after surgery. In these dogs, ATT, FBA and FA data were used, in addition to Doppler ultrasound (DUS) results, to determine the degree of closure of the shunt. If the PSS could still be visualised 1 month post-surgery, a second evaluation was performed 2 months later.

To assess the diagnostic value of the different tests for screening purposes in purebred dog populations (screening group), the positive and negative predictive values of the ATT, FBA and FA were calculated based on the established sensitivity and specificity of the tests for CPSS in the symptomatic group, and an estimated prevalence range (1–5%) of CPSS in purebred dogs (Tobias and Rohrbach, 2003).

Diagnostic tests

PSS was suspected with appropriate clinical signs and increased baseline FA or FBA concentrations, as previously described (Ruland et al., 2010). Confirmation and localisation of PSS was made through visualisation of the shunt(s) by either DUS, contrast computed tomography (CCT), surgery or post-mortem examination. PSS was excluded when no shunting was visualised by CCT or at post-mortem examination. PSS was also excluded when shunting was not visible by DUS and a diagnosis other than PSS (that could entirely explain all the clinical signs) was confirmed. Primary portal vein hypoplasia (PPVH) was diagnosed using liver histology in combination with an absence of evidence for other portovenous anomalies (e.g. CPSS, arterioportal fistula, APF) on DUS or post-mortem examination. APF was diagnosed by DUS.

Parenchymal liver diseases were diagnosed by histological examinations. Liver biopsies were taken either percutaneously using a 14-16G Tru-cut biopsy needle under ultrasonographic guidance (Rothuizen et al., 2006), or wedge biopsies obtained at post-mortem. Cytological diagnosis was included in the study if the findings were sufficient to confirm a definitive diagnosis. Fine needle aspiration biopsies from the liver were collected under ultrasound guidance using a 22G needle. In dogs belonging to group 2 (PSS–), with non-hepatic diseases, a definitive diagnosis was made according to standard diagnostic procedures.

The ATT was performed as previously described (Rothuizen and Van Den Ingh, 1982). Blood was drawn just before (T0) and at 20 (T20) and 40 (T40) min after a rectally applied ammonium chloride solution (5% w/v, 2 mL/kg bodyweight) and collected in Na–EDTA tubes that were kept on ice until the assay was performed. ATT results were considered abnormal if FA were above the reference range (15–45 μmol/L) at 20 or 40 min after ammonium chloride administration.

Blood sampling and assay methods

Venous blood samples were collected after a 12 h fasting period. Blood samples were collected from the jugular vein. For FA measurements, blood was placed in Na–EDTA tubes, which immediately were placed on melting ice. FA measurements were within 10 min of collection by a microdiffusion method using PocketChem (Menarini Diagnostics). FBA concentrations were determined by a commercially available enzymatic colorimetric test kit (Diazyme Laboratories) with the UniCel DxC 600 assay (Beckman Coulter). The reference range used for FBA was 0–10 μmol/L.

Statistical analysis

Sensitivity, specificity, PPV, NPV, LR+, LR– and overall accuracy of the ATT, FBA and FA for the diagnosis of PSS were determined for the symptomatic dogs and the CPSS post-operative control group dogs using a 2 × 2 classification (contingency) table. The same parameters were also calculated for CPSS in the symptomatic dog population. Predictive values were calculated using the following formulae based on Bayes' theorem (Gardner and Greiner, 2006):

$$\text{PPV} = \text{sensitivity} \times \text{prevalence} / ([\text{sensitivity} \times \text{prevalence}] + [1 - \text{specificity}] \times [1 - \text{prevalence}]).$$

$$\text{NPV} = \text{specificity} \times (1 - \text{prevalence}) / ([1 - \text{sensitivity}] \times \text{prevalence} + \text{specificity} \times [1 - \text{prevalence}]).$$

For the screening population, the predictive values were calculated at two prevalence rates, namely, 1% and 5%. Likelihood ratios were calculated for the likelihood of a true positive (LR+) and a true negative test (LR–), as previously described (Akobeng, 2007). The 95% confidence intervals for the proportions and likelihood ratios were calculated as previously described (Bulpitt, 1987; Simel et al., 1991). Overall accuracy was defined as the sum of the true positives plus the true negatives divided by the total number of dogs tested (Alberg et al., 2004).

The same diagnostic parameters were also calculated for serial testing for the combinations of FBA+FA, FA+FBA, ATT(T40) + FBA and FBA+ATT(T40). Tests were considered positive only if both test results were positive. Calculations were made using the following formulae:

$$\text{Sensitivity of test A+/B+} = \text{Sensitivity test A} \times \text{Sensitivity test B}.$$

$$\text{Specificity of test A+/B+} = \text{Specificity test A} + (1 - \text{Specificity test A}) \times \text{Specificity test B}.$$

Predictive values and likelihood ratios were calculated in the manner described earlier.

Results

Medical records of 1336 dogs were analysed. Two hundred and seventy-one dogs met the inclusion criteria. In 133 (49%) dogs, a definitive diagnosis of PSS was established (PSS+) and in 138 (51%) dogs PSS was excluded (PSS–). Table 1 summarises the type of shunting, the organ system to which clinical signs were attributed, and the diagnostic tests used to confirm the diagnosis in each group. In four dogs, which were presented with clinical signs suggestive of PSS and with increased FBA and FA, visualisation of PSS was made using CCT. Two of those dogs were diagnosed with APSS and two with CPSS.

Fifty-seven of the dogs diagnosed with CPSS underwent surgery. Fifty-three dogs returned for post-surgical evaluation (CPSS post-operative control group). During post-surgical follow-up, an ATT was performed in 49 cases and FBA and FA concentrations were determined in 40 cases. In 90% (120/133) of the dogs in the PSS+ group, CPSS was confirmed. PPVH and APF were diagnosed in five and three dogs, respectively. Acquired PSS as a result of chronic hepatitis or liver cirrhosis was diagnosed in five dogs. In the PSS– group, dogs with liver diseases represented the largest subgroup (41%, 57/138) followed by diseases related to the central nervous system (22%, 30/138) and gastrointestinal diseases (15%, 21/138; Table 2). Diseases diagnosed in the subgroup CNS were intracranial neoplastic or inflammatory diseases, hydrocephalus, granulomatous meningoencephalitis, and intra cranial bleeding. The diseases diagnosed in the subgroup gastrointestinal diseases included lymphoplasmacytic enteritis, ileus, gastrointestinal neoplastic diseases and pancreatitis. The subgroup 'other' consisted of five dogs with PU/PD as the sole clinical sign; these dogs were diagnosed with primary polydipsia. There were six other dogs in which no diagnosis could be made, but PSS could be excluded by means of DUS in combination with either an abdominal CT or liver histological examination.

Table 1
Diseases causing portosystemic shunting (PSS) in dogs with PSS (PSS+, group 1) and organ systems involved in dogs without PSS (PSS–, group 2), with diagnostics used to confirm the diagnosis.

Population	Group	*n*	Disease/organ system	*n*	Diagnostic test, *n*						
					US	HIS	CT	MRI	LAP	PME	Cytology
Symptomatic dogs	1. PSS+	133	CPSS	120	113		4		57	3	
			PPVH	5	1	5	1				
			APF	3	3		1				
			CH/liver cirrhosis	5	5	5					
	2. PSS–	138	Liver	57	55	54	7			7	3
			Gastrointestinal	21	21	8	3		1		
			Endocrine	14	14	2	4			1	
			Cardiology	4	4						
			Kidney	1	1	1					
			CNS	30	22	14	2	12		15	2
			Other	11	5	4	2			1	
CPSS control dogs	1. PSS+	25	CPSS		25		4				
	2. PSS–	28			28		4				

APF, arterioportal fistula; CH, chronic hepatitis; CPSS, congenital portosystemic shunt; CT, computed tomography; HIS, histology; LAP, laparotomy; MRI, magnetic resonance imaging; PME, post-mortem examination; PPVH, primary portal vein hypoplasia; US, ultrasound.

Diagnostic performance of the ATT, FBA and FA concentrations and the combination of these tests for diagnosing PSS in symptomatic dogs are shown in Table 3. No adverse events were reported during or after performing the ATT. The prevalence of PSS in the group in which the ATT was performed was 40% (19/47). The T40 was not performed in 4/47 dogs. In 8/19 dogs with PSS, the baseline FA concentration was within the reference range. FBA and FA concentrations were measured in 254 dogs. The prevalence of PSS in that group was 50% (127/254). All diagnostic parameters for the ATT at T40 were higher than at T20, except for LR– (Table 3). The ATT at T40 had the highest sensitivity (100%) and NPV of all diagnostic tests assessed. If both FBA and FA concentrations were increased above reference values on serial testing, there was a relatively low sensitivity (87%) but the highest specificity (97%), PPV (97%), and LR+ (29).

For the CPSS post-operative control group (Table 4), all diagnostic parameters for the ATT T40 were higher than for the ATT T20, except for the LR–, which was lower. The sensitivity of all tests was lower and the specificity was higher in the CPSS post-operative control group than the symptomatic group, except for FBA+/ATT+. FA and FBA+/FA+ had the highest specificity and PPV (both 100%), but the overall accuracy of FA was higher (65%) than FBA+/FA+ (53%).

Across the board test sensitivity and specificity for the detection of CPSS in symptomatic dogs were almost identical to those calculated for the PSS group (Table 5). The only difference was an increase in sensitivity of FA from 88% to 90% the PSS group. When the estimated prevalence values of CPSS in purebred dogs (1% and 5%) were taken into consideration, the PPVs of all tests decreased from ranges between 64 and 97% in the symptomatic dog group (Table 3) to values varying from 11 to 61% (5% prevalence) and from 2 to 17% (1% prevalence; Table 5). The NPVs of all tests increased to values of 100% under these conditions.

Table 2
Diagnosis in dogs affected by liver disease without portosystemic shunting (PSS).

Diagnosis	*n*
CH + cirrhosis	11
Reactive hepatitis	11
CH	11
SIH	3
Lymphoma	3
Adenocarcinoma	5
Hepatoma	1
Subacute hepatitis	2
Acute hepatitis	2
PPVH	3
Common bile duct obstruction	2
Bile bladder mucocele	2
Cholangioma	1

CH, chronic hepatitis; PPVH, primary portal vein hypoplasia; SIH, steroid induced hepatopathy.

Discussion

In this study, the test performance of the ATT, FBA and FA for the diagnosis of PSS was evaluated by calculating the sensitivity, specificity, predictive values, likelihood ratios, and accuracy of the

Table 3
Sensitivity, specificity, predictive values, likelihood ratios and overall accuracy of the ammonia tolerance test (ATT), fasting bile acid concentration (FBA), fasting ammonia concentration (FA) and combined serial testing for the diagnosis of portosystemic shunts in symptomatic dogs, using laboratory reference ranges as cut-off values.

Analyte	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+	LR–	Overall accuracy (%)
ATT							
T20	95 (73–99)	65 (44–81)	64 (44–81)	95 (74–99)	2.6 (1.6–4.4)	0.08 (0.01–0.5)	77
T40	100 (78–100)	79 (59–91)	72 (47–87)	100 (84–100)	4.7 (2.3–9.5)	0.00	85
FBA	98 (93–99)	58 (49–67)	70 (63–77)	96 (89–99)	2.3 (1.9–2.9)	0.04 (0.01–0.13)	68
FA	88 (81–93)	90 (83–94)	90 (83–94)	88 (81–93)	8.6 (5.1–14.4)	0.13 (0.1–0.2)	65
FBA+/FA+[a]	87 (79–92)	97 (91–99)	97 (91–99)	86 (80–94)	29 (9.5–89)	0.13 (0.1–0.2)	92
FA+/FBA+[a]	80 (71–87)	96 (90–99)	95 (88–99)	83 (75–89)	20 (7.6–52)	0.21 (0.14–0.31)	88
ATT (T40)+/FBA+[a]	98 (97-95)	91 (90–91)	91 (90–92)	98 (97–98)	10.6 (9.9–11.2)	0.02 (0.02–0.03)	94
FBA+/ATT (T40)+[a]	98 (92–100)	91 (84–96)	92 (85–96)	98 (92–100)	10.9 (5.8–20.3)	0.02 (0.01–0.1)	95

ATT+/FBA+ or FBA+/ATT+, serial testing starting with ATT or FBA, respectively; CI, confidence intervals; FBA+/FA+ or FA+/FBA+, serial testing starting with FBA or FA, respectively; LR+, positive likelihood ratio; LR–, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; T20 and T40, 20 and 40 min after intrarectal ammonium chloride administration (2 mL/kg), respectively.

[a] In serial testing, the test was considered positive when the first and the second analyte were positive.

Table 4
Sensitivity, specificity, predictive values, likelihood ratios and overall accuracy of the ammonia tolerance test (ATT), fasting bile acid concentration (FBA), fasting ammonia concentration (FA) and combined serial testing for the diagnosis of portosystemic shunts (PSS) in the congenital PSS post-operative control group, using laboratory reference ranges as cut-off values.

Analyte	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+	LR–	Overall accuracy (%)
ATT							
T20	86 (67–95)	76 (52–92)	83 (64–94)	80 (56–94)	3.6 (1.6–7.8)	0.19 (0.07–0.48)	61
T40	89 (70–97)	85 (62–96)	89 (70–97)	85 (62–96)	5.9 (2.1–17.0)	0.13 (0.04–0.39)	87
FBA	68 (46–85)	67 (38–88)	77 (54–92)	56 (30–78)	2.04 (0.95–4.3)	0.48 (0.24–0.94)	67
FA	44 (24–65)	100 (78–100)	100 (71–100)	52 (32–70)	0.00	0.56 (0.40–0.79)	65
FBA+/FA+	24 (9–45)	100 (78–100)	100 (54–100)	44 (27–62)	0.00	0.76 (0.61–0.95)	53
ATT+/FBA+	60 (36–78)	73 (45–92)	79 (54–94)	52 (30–74)	2.25 (0.92–5.52)	0.55 (0.31–0.96)	65
FBA+/ATT+	8 (1–26)	93 (68–99)	67 (12–95)	35 (22–55)	1.20 (0.12–12.13)	0.99 (0.83–1.18)	40

ATT+/FBA+ or FBA+/ATT+, serial testing starting with ATT or FBA, respectively; CI, confidence intervals; FBA+/FA+ or FA+/FBA+, serial testing starting with FBA or FA, respectively; LR+, positive likelihood ratio; LR–, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; T20 and T40, 20 and 40 min after intrarectal ammonium chloride administration (2 mL/kg), respectively.

tests in 271 dogs. Additionally, the usefulness of these tests as 'screening tools' for detecting CPSS in clinically healthy purebred dogs was evaluated by calculating the predictive values using the estimated prevalence of CPSS in such populations.

The ideal test for diagnosing canine PSS would be one that detects or excludes PSS with maximum accuracy. The prevalence of PSS in the general symptomatic dog population in this study was 50%; the purpose of testing for PSS in this population should emphasise 'ruling in' or 'ruling out' PSS (i.e. confirmatory testing). The results of this study show that no single test is sufficient for both detecting and excluding PSS. The ATT and FBA are much more sensitive than FA concentrations for detecting PSS, but lack the high specificity of increased FA concentrations.

As single tests, the ATT and FBA are mainly useful for excluding PSS in symptomatic dogs. The ATT T40 was the most sensitive test (100%) for detecting PSS, and had a NPV of 100%. Therefore, normal FA concentrations at T40 can rule out PSS. Similarly, PSS would also be very unlikely in a symptomatic dog that had FBA in the normal reference range. As both the ATT and FBA had relatively low PPVs and LR+s, increased FA concentrations at T40 or increased FBA would not be sufficient to 'rule in' PSS. Additional diagnostics would therefore be required to visualise or exclude possible PSS.

Serial testing which combines FBA and FA or ATT and FBA offers the advantage of increasing specificity, PPV and LR+. In the symptomatic population, serial testing starting with FBA and following with FA had the highest specificity, PPV and LR+ when both FBA and FA were increased. The high values of the PPV and LR+ (97% and 29%, respectively) effectively 'rule in' PSS when both concentrations are increased. PPV and LR+ were slightly lower (95% and 20, respectively) when FA was measured first and followed by FBA, but were still sufficient to make PSS very probable when both concentrations were increased. Serial testing with ATT and FBA could also be helpful in discriminating PSS from other diseases that typically present with similar clinical signs. However, the specificity and PPV of such serial testing were lower than when FBA and FA concentrations were used.

In this study, the sensitivity, specificity and likelihood ratios of FBA and FA were comparable to those reported by Ruland et al. (2010); that study also demonstrated that FBA was more sensitive but less specific than FA for the detection of PSS in dogs. However, Gerritzen-Bruning et al. (2006) reported higher sensitivity for FA than FBA. The reason for this difference is not clear; however one explanation could be the different diagnostics used for the confirmation and exclusion of PSS.

Our study protocol used the diagnostic ultrasonographic protocol developed and published by Szatmari et al. (2004). Using this protocol reportedly leads to detection of 100% of PSS cases. Another study reported that the sensitivity, specificity, PPV and NPV of ultrasonography for the identification of PSS were 92%, 98%, 98%, and 89%, respectively (d'Anjou et al., 2004). Therefore, visualisation of shunting using DUS was sufficient to confirm the diagnosis of PSS in our study. In four cases in which visualisation of PSS was not possible using DUS, PSS was confirmed by CCT. CCT has been reported to be superior to abdominal ultrasonography for the detection of PSS in dogs (Kim et al., 2013).

Sensitivity and specificity were recalculated for the CPSS post-operative control population because the degree of shunting was significantly reduced in most dogs after surgery. Therefore, these

Table 5
Sensitivities, specificities, predictive values, likelihood ratios and overall accuracy of the ammonia tolerance test (ATT), fasting bile acid concentration (FBA), fasting ammonia concentration (FA) and combined serial testing for the diagnosis of congenital portosystemic shunts (CPSS) in a population where the prevalence of CPSS was estimated at either 1% or 5% (screening population), using laboratory reference ranges as cut-off values.

Analyte	Prevalence	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+	LR–	Overall accuracy (%)
ATT								
T40	0.05	100 (78–100)	79 (78–80)	20 (18–22)	100 (99–100)	4.76 (4.58–4.95)	0.00	80
T40	0.01	100 (78–100)	79 (78–80)	4.6 (4–6)	100 (99–100)	4.76 (4.58–4.95)	0.00	80
FBA	0.05	98 (93–99)	58 (49–67)	11 (10–12)	100 (99–100)	2.33 (2.27–2.40)	0.03 (0.02–0.06)	60
FBA	0.01	98 (93–99)	58 (49–67)	2 (2–3)	100 (99–100)	2.33 (2.25–2.42)	0.03 (0.01–0.14)	60
FA	0.05	88 (81–93)	90 (83–94)	32 (29–34)	99 (99–100)	8.8 (8.22–9.42)	0.13 (0.11–0.17)	90
FA	0.01	88 (81–93)	90 (83–94)	8 (7–10)	100 (99–100)	8.8 (8.01–9.66)	0.13 (0.08–0.23)	90
FBA+/FA+	0.05	86 (73–94)	97 (96–98)	61 (48–72)	99 (98–100)	29.1 (20–42.7)	0.14 (0.1–0.3)	97
FBA+/FA+	0.01	86 (79–92)	97 (95–96)	17 (14–21)	100 (99–100)	20.5 (18.1–23.1)	0.15 (0.1–0.2)	97
ATT+/FBA+	0.05	98 (96–99)	90 (90–91)	36 (33–38)	100 (99–100)	10.6 (9.9–11.3)	0.02 (0.01–0.04)	91
ATT+/FBA+	0.01	98 (92–99)	90 (90–91)	10 (8–12)	100 (99–100)	10.6 (9.9–11.3)	0.02 (0.01–0.09)	91
FBA+/ATT+	0.05	98 (96–99)	91 (90–91)	36 (33–38)	100 (99–100)	10.6 (9.9–11.3)	0.02 (0.01–0.04)	91
FBA+/ATT+	0.01	98 (93–100)	91 (90–91)	10 (8–12)	100 (99–100)	10.6 (9.9–11.3)	0.02 (0.01–0.09)	91

ATT+/FBA+ or FBA+/ATT+, serial testing starting with ATT or FBA, respectively; CI, confidence intervals; FBA+/FA+ or FA+/FBA+, serial testing starting with FBA or FA, respectively; LR+, positive likelihood ratio; LR–, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; T20 and T40, 20 and 40 min after intrarectal ammonium chloride administration (2 mL/kg), respectively.

values cannot be compared to those of the symptomatic dog population. Surgical attenuation of the shunt resulted in a substantial decrease in sensitivity and an increase in specificity for all tests in comparison to the symptomatic dog population. In some dogs where surgical attenuation led to clinical recovery, centrifugal flow could still be visualised in the attenuated shunt using DUS. The presence of this functional but clinically irrelevant PSS is the most probable explanation for the decrease in sensitivity and increase in specificity in all tests.

We suggest that the initial test to evaluate the effects of surgical attenuation of CPSS should be FA concentration. If it is increased, PSS is still present; if it is normal, an ATT could be performed. If the ATT is abnormal, the probability of PSS in our study population was 89%, whereas if the ATT was normal, PSS could be excluded with a probability of 85%. In both of these cases, DUS should be performed to visualise or exclude PSS; therefore the need to perform an ATT or other additional blood tests after obtaining a normal FA concentration is questionable. This finding is in contrast to a previous study (Berent and Tobias, 2009), which suggested that ATT results could be used as a semiquantitative guide to the degree of PSS and the post-surgical attenuation of the shunt.

For the purposes of screening as a breeding strategy, a sensitive, practical and cheap diagnostic test is desirable. The ATT offers a sensitivity of 100%, but in comparison with baseline FA or FBA is less practical. In a population where CPSS occurs at a prevalence <5%, the NPV of all tests becomes 100%. The results of our study suggest that FBA concentrations are the most useful test for screening for CPSS in a healthy population. The sensitivity of FBA was high and comparable with the ATT T40, sample handling does not require extra measures, and samples can also be sent to a commercial laboratory for analysis. With a NPV of 100% (i.e. FBA within the normal reference range) a negative screening test result virtually excludes CPSS. A positive result has a very low PPV and additional tests would be required to diagnose CPSS (e.g. DUS or other diagnostic modalities).

Although the diagnostic accuracy of the serial testing performed in this study was very high in the screening population, these values are unreliable at low prevalences (Alberg et al., 2004). Additionally, the PPVs and LRs of the serial testing did not differ considerably from the values achieved when tests were performed in isolation. Because of this, serial testing using combinations of ATT, FBA and FA cannot be recommended for screening for canine CPSS.

In this study, no adverse events were reported in any of the dogs during or after the ATT. Adverse events such as vomiting, hypersalivation and lethargy have been reported in association with the ATT (Strombeck et al., 1975; Rothuizen and Van Den Ingh, 1982), but they occurred when ammonium chloride was administered orally, rather than rectally, as described here. Therefore, we conclude that the test is safe to perform using ammonium chloride per rectum, including in dogs suspected of PSS. Additionally, in our study, T40 had diagnostic superiority in all respects to T20, making T20 redundant. Consequently, we suggest limiting the ATT to two blood samples at T0 and T40. This modification simplifies the ATT, makes it more applicable for clinical use and improves animal welfare.

Conclusions

In symptomatic dogs, an increase in both FBA and FA is sufficient for diagnosing PSS. In these dogs, diagnostic imaging (DUS or CCT) can confirm the diagnosis, but more importantly, can determine the type of PSS, thereby informing prognosis. PSS in symptomatic dogs can be virtually excluded when FBA is not increased. In these cases, or in cases where the FA is within the reference range, a normal ATT could rule out PSS completely. Measurement of FA is the testing method of choice for diagnosing PSS in dogs presented for post-surgical evaluation. In this population, increased FA indicates PSS. Either DUS or CT should be performed if FA or FBA are not increased to confirm or rule out PSS. For screening purposes, a FBA in the reference range is sufficient to exclude a diagnosis of PSS.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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